

Instructions for Use

ANAEROBIC DIFFERENTIATION DISKS

Cat. no. Z8411	Colistin	50 disks/cartridge
Cat. no. Z7191	Kanamycin	50 disks/cartridge
Cat. no. Z7501	Vancomycin	50 disks/cartridge

INTENDED USE

HardyDisk™ Anaerobic Differentiation Disks (also referred to as "special-potency disks") are used to verify gram stain reactions of anaerobic bacteria, and are especially helpful with certain clostridia that tend to stain gram-negative. They are useful in separating anaerobic, gram-negative bacilli into several groups, aiding in their presumptive identification. These disks can also differentiate *Fusobacterium* species from other true gram-negative bacilli.^(2,3,6,7)

SUMMARY

Special-potency antimicrobial disks of colistin, kanamycin, and vancomycin can be used as an aid in clarifying gram stain reaction of anaerobes, and in preliminary grouping of anaerobic gram-negative bacilli. In general, gram-positive organisms are resistant to colistin and susceptible to vancomycin, while most gram-negative organisms are resistant to vancomycin.^(3,7,10) Most anaerobes have a characteristic susceptibility pattern to these special-potency, high concentration disks. Therefore, most clinically significant anaerobic gram-negative rods can be separated into broad groups, based on relatively few tests. For example, the *B. fragilis* group and *Porphyromonas* species can be presumptively identified by their disk patterns and a few other simple tests (such as; indole, catalase, esculin, etc.).⁽²⁾

The use of these antibiotic susceptibility patterns to aid in the identification of anaerobic bacteria was first reported in 1967 by Finegold, et al. In this study, high concentration disks were used to differentiate between the main groups of gram-negative anaerobic bacteria. It was determined that the susceptibility tests were rapid and easy to perform.^(8,9)

Complete identification of anaerobes can be costly, and often requires various biochemical tests, GLC (gas liquid chromatography), etc. Many clinical labs do not perform complete identification of anaerobes, since presumptive identification can be just as useful in determining the appropriate therapy.⁽³⁾ HardyDisk™ Anaerobic Differentiation Disks are helpful in the presumptive identification of these organisms. Due to the high concentrations of these antibiotics, these disks are not intended for use in determining antibiotic susceptibility for therapeutic purposes.

FORMULA

Each HardyDisk™ Anaerobic Differentiation Disk is prepared by impregnating the below specified concentration of the appropriate antibiotic onto a 6mm diameter high quality filter paper disk.

Colistin	10mcg
Kanamycin	1,000mcg
Vancomycin	5mcg

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20 to +8°C. away from direct light. The disks should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Specimen Collection: This product is not intended for the primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

If testing is performed on the open bench, all plates should be promptly (within 20 minutes) incubated anaerobically. Some clinical isolates may die after relatively short oxygen exposure.^(3,7)

1. Allow disks to equilibrate to room temperature.
2. Select one, well-isolated colony of an anaerobic organism to be tested from a primary or pure subculture.
3. Inoculate a non-selective anaerobic blood agar plate Brucella with Hemin and Vitamin K (Cat. no. A30), by streaking the first quadrant back and forth several times, to ensure an even, heavy lawn of growth. Streak the other quadrants for isolation.
4. Using forceps, place the colistin, kanamycin, and vancomycin disk (one each) in the first quadrant well apart from each other (nitrate, SPS, and bile disks may be placed in the second quadrant of the plate at this time, refer to listed references for procedure information).^(3,7)
5. Incubate the plates anaerobically at 35-37°C. for 24-48 hours, or until sufficient growth is observed. Longer incubation time may be required for some organisms.

INTERPRETATION OF RESULTS

Sensitive (S) - Zone of inhibition is greater than or equal to 10mm.

Resistant (R) - Zone of inhibition is less than 10mm.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The definitive identification of most species requires some additional biochemical testing.⁽²⁾

Rare strains of *B. fragilis* are susceptible to colistin.⁽³⁾

When using these special-potency disks for rapid identification, it is important to remember that the results of the test does not give information about the antimicrobial agents that can be used for therapy.^(6,9)

Occasionally, some clostridia and lactobacilli can appear resistant to vancomycin (5ug).⁽⁷⁾

Porphyromonas species are gram-negative bacilli, but are susceptible to vancomycin.⁽⁷⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, disk dispensers, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

End users should anticipate the following typical performance characteristics when testing quality control.

Test Organisms	Inoculation Method*	Incubation			Results		
		Time	Temperature	Atmosphere	Colistin	Kana	Vanco
<i>Fusobacterium nucleatum</i> ATCC® 25586	*	48-72hr	35°C	Anaerobic	S	S	R
<i>Bacteroides fragilis</i> ATCC® 25285	*	48-72hr	35°C	Anaerobic	R	R	R
<i>Clostridium perfringens</i> ATCC® 13124	*	48-72hr	35°C	Anaerobic	R	S	S

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

HardyDisk™ Anaerobic Differentiation Disks are 6mm (in diameter) filter paper disks and should appear white in color.

The disks can be identified by a letter code printed on both sides of the disk. The Colistin disk (Cat. no. Z8411) is identified by the letters CT, the Kanamycin disk (Cat. no. Z7191) has the letters KAN, and Vancomycin (Cat. no. Z7501) has Va5 printed on both sides of the disk.



Showing Vancomycin Resistance

Bacteroides fragilis (ATCC® 25285) growing on Brucella Agar with Hemin and Vitamin K (Cat. no. A30) with a Vancomycin disk (Cat. no. Z7501). Incubated anaerobically for 48 hours at 35°C.



Showing Vancomycin Sensitivity

Clostridium perfringens (ATCC® 13124) growing on Brucella Agar with Hemin and Vitamin K (Cat. no. A30) with a Vancomycin disk (Cat. no. Z7501). Incubated anaerobically for 24 hours at 35°C.

REFERENCES

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